Trends in Parasitology

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Review

Pharma to farmer: field challenges of optimizing trypanocide use in African animal trypanosomiasis

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Trypanocides are a key control component of African animal trypanosomiasis (AAT) in tsetse-infested areas of sub-Saharan Africa. While farmers are dependent upon trypanocides, recent research highlights their inappropriate and ineffective use, problems with drug quality, and treatment failure. There are currently gaps in knowledge and investment in inexpensive AAT diagnostics, understanding of drug resistance, and the effective use of trypanocides in the field. Without this important knowledge it is difficult to develop best practice and policy for existing drugs or to inform development and use of new drugs. There needs to be better understanding of the drivers and behavioural practices around trypanocide use so that they can be incorporated into sustainable solutions needed for the development of effective control of AAT.

Highlights

Control of African animal trypanosomiasis (AAT) is hampered by limited diagnostics, inappropriate trypanocide use, poor drug quality, and drug resistance.

The scope and quality of current literature on AAT incidence, control, and resistance does not allow for robust comparisons or assessment of the validity of extrapolating to other populations.

A united effort is needed to address AAT at local, national, and international settings to ensure a greater chance for success.

AAT control programmes must be sustainable through funding, cross-sectoral engagement, and fostering sustainable behavioural change through incentives and accountability.

Obstacles to improving trypanocide use in AAT

African animal trypanosomiasis (**AAT**) (see Glossary) is an important constraint on livestock production in parts of sub-Saharan Africa (SSA) where the tsetse fly vector is found. Improving AAT control is important for development and food security, whilst potential impacts on the risk of human African trypanosomiasis (HAT) highlight its value as a **One Health** intervention [1–3]. Trypanocides (Box 1) are a key control component of AAT. Whilst a recent review [4] described antitrypanosomal chemotherapy, there is an urgent need to consider our understanding of how these widely available drugs are used in the field. This is needed to improve effective use of existing drugs; it is timely because these issues are relevant to the implementation of new trypanocides that are under development. This paper reviews recent literature on trypanocide usage, effectiveness, and resistance, and identifies barriers to optimal use in order to make recommendations regarding future trypanocide use.

The role of trypanocides in AAT control

The direct and indirect control options for AAT are summarized by Meyer *et al*. [5] in their systematic review. Trypanocides are used for the prevention and treatment of infection in individual or groups of cattle, whilst tsetse control includes the aerial- or ground-based application of insecticide to the resting sites of tsetse, or baits (**targets** or **insecticide-treated cattle**) to attract and kill tsetse (Figure 1). Despite significant investment in tsetse control, in 2001 it was estimated that vector-control operations covered only <2% of tsetse-infested areas [6].

However, livestock keepers often adopt vector-control methods. For instance, in parts of Tanzania [2], Zimbabwe [7], and Uganda [8,9], there is widespread treatment of cattle with pyrethroids to control ticks and tsetse. Whilst livestock keepers usually treat their animals to protect their own herd, insecticide treatment of cattle is proven in its ability to reduce tsetse populations

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Box 1. Trypanocides used to treat and prevent AAT in ruminants

There are three trypanocides available for use in sub-Saharan Africa for AAT in ruminants: diminazene aceturate (DZ), isometamidium chloride (ISM), and homidium salts (HM – also known as ethidium bromide). While a range of doses is described in the literature for these medications, on the packaging for retail sale there is typically only one dose listed.

DZ was introduced in 1955 for use in livestock and is used as a curative therapy. It is administered by intramuscular (IM) or subcutaneous (SC) route at a dose of 3–5 mg/kg [4]. DZ is usually sold in single-dose sachets of powder that must be diluted with sterile water.

ISM was introduced in the 1960s and is given via deep IM injection for treatment or prophylaxis at 0.25–1 mg/kg [4]. It is the only drug labelled for prophylaxis of AAT. Administration is associated with lesions at the site of IM injection, especially with long-term use. If used IV, the prophylactic effects are negated, whereas with IM administration it results in a 2–6 month prophylactic period. ISM is sold in a multi- or single-dose sachet of powder that must be diluted with sterile water.

HM was introduced in 1952 and is given by deep IM in cattle or IV in small ruminants at 1 mg/kg for treatment [4]. It is known for its proven mutagenic and possible carcinogenic risk and its use is discouraged [4]. HM does have some prophylactic properties but less so than ISM. HM is sold in a tablet form that must be ground and diluted with sterile water.

Quinapyramine was used in cattle between 1950 and 1970 but was removed from the market due to toxicity and resistance [4]. Despite this, it is available for use in several species, including cattle[iii,iv], although the extent of its use is not clear.

and hence reduce the incidence of AAT [10] if applied to enough cattle at relatively large scale and with even coverage [11]. For example, one model evaluating the control of AAT with insecticides found that a minimum of 20% of cattle must be treated over a relatively large area (>100 km^2) for effective control, whereas individual animals may be treated with trypanocides to protect or cure them [11]. Similarly, Shaw *et al*. [12] evaluated the cost benefit of control methods and found that only when there is sufficient cattle density (>10 cattle/km^2) is insecticide use profitable.

Despite insecticide use, most livestock keepers regard trypanocides as the primary option for AAT control. In Tanzania, it is reported that 0–45% of farmers, depending on district, were aware that tsetse control could control AAT, whilst up to 60% of farmers reported using chemotherapy as a control method for AAT [13]. In several countries, for example, Togo [14], AAT control is almost

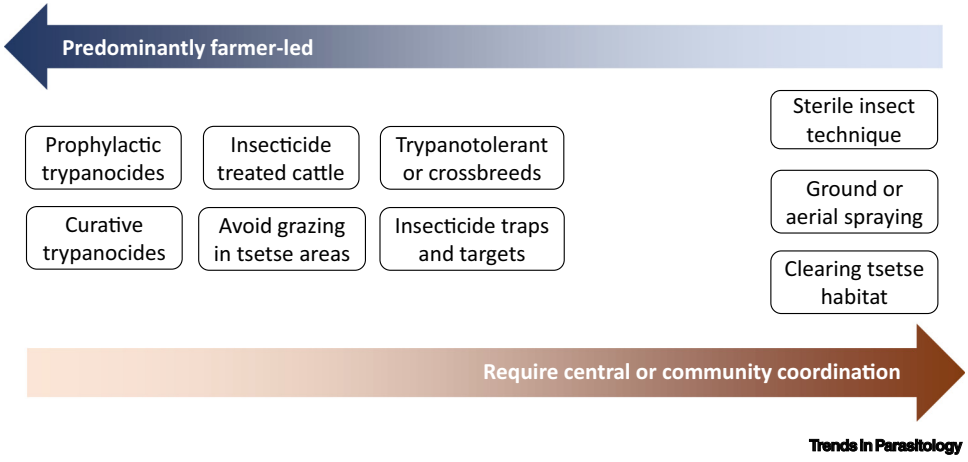

Figure 1. Control options for African animal trypanosomiasis (AAT) highlighting farmer versus community or centrally required coordination. Adapted from [5]. Control measures for AAT are highlighted, with predominantly farmer-led methods indicated by the upper arrow, where items to the left of the figure are more easily implemented and utilized by individual farmers. Predominantly centrally or community-led control measures are indicated by the lower arrow, where items to the right of the figure are more easily and effectively implemented by central bodies such as government agencies or communities.

Glossary

African animal trypanosomiasis (AAT): a disease of domestic animals caused by parasitic protozoa of the genus *Trypanosoma* across sub-Saharan Africa. Cattle, sheep, and goats are infected by strains of *T. congolense, T. vivax,* and *T. brucei*. Human African trypanosomiasis (HAT) is a disease of humans, caused by *T. brucei rhodesiense* and *T. brucei gambiense,* and it is zoonotic. AAT and HAT are transmitted by the tsetse fly (*Glossina* spp.) vector, and *T. vivax* can also be transmitted by biting flies. Trypanosomes have highly variable surface antigens that regularly change, making antibody responses ineffective, leading to persistent infections, and preventing the development of vaccines. Clinical signs in ruminants include anaemia, poor body condition, intermittent fever, poor appetite, dull/staring coat (rough and upstanding appearance to hair), lymph node enlargement, abortion, reduced milk yield, diarrhoea, dehydration, and increased lachrymation. Cattle typically have chronic infections, which can include high mortality, especially if already in poor condition or stressed.
Antimicrobial resistance (AMR) or resistance: the characteristic of those parasites, bacteria, viruses, or fungi which no longer respond to medications (like trypanocides) used to manage associated infections, due to an intrinsic change in which the pathogen lowers its sensitivity to a drug. This is one way in which treatment failure occurs.
Insecticide-treated cattle: cattle which have been treated with synthetic pyrethroids, such as alphacypermethrin and deltamethrin, that are applied by spraying, dipping, or pour-on methods; these agents can kill tsetse flies. Spraying and dipping require more infrastructure or equipment than the pour-on approach. The frequency of application depends on how long the insecticide remains active on animals. In field settings, insecticides are applied as often as weekly.
One Health: the interaction between animal, human, and environmental health as it pertains to collaborative approaches to improving cross-sectoral health outcomes.
Progressive control pathway (PCP): a five-stage stepwise approach to reduce, eliminate, and eradicate a disease. Stages include preparatory

exclusively based on chemotherapy, without insecticide use or other tsetse-control approaches. It is clear that trypanocides will remain a fundamental part of AAT control.

Quantifying trypanocide use in the field

The trypanocide (Box 1) market comprises both formal and informal sectors; HealthforAnimals, formerly the International Federation for Animal Health, estimates that the trade of unregistered and substandard veterinary drugs in Africa is valued at US$400 million annually, which is estimated to be of similar value to the official market[i]. The use of trypanocides is hard to enumerate and robust published data are rare. Estimates of annual usage of trypanocides range in SSA from 35 million doses to 50 million doses [15–17] with a total value of US$20 million [18] to US $90 million [19,20]. Notably, although these estimates are very widely cited, none of these references provide methodology, making it impossible to assess their robustness or to consider likely trends over time. Country-level estimates of the trypanocide market are rare, but US$8.4 million was spent on trypanocides annually in southwest Nigeria, representing the largest class of purchased cattle drugs [21,22]. A more accurate method to determine trypanocide use would be to evaluate records of import of veterinary drug suppliers, as was done in Cameroon for antibiotics in livestock; however, there are no similar reports in the literature on trypanocides [23].

Individual dose costs for trypanocides in Nigeria have been estimated at US$2–3.55, whilst across SSA the cost of single doses of drug are estimated to fall between US$0.50 and US$3.00 [19]. In Uganda, the cost of a single dose of both diminazene aceturate (DZ) with added vitamin B12, and isometamidium chloride (ISM) for a 192 kg cow was US$0.64 and US$1.00 respectively, and US$1.42 and US$1.78 inclusive of required supplies and delivery of injection respectively [24]. Holt *et al*. [25] surveyed livestock owners across five SSA countries to identify the costs of trypanocides used and reported that 16–63% of livestock owners spent more than US$55/2 years, depending on location, herd size, and cattle breed (**trypanotolerant** vs. susceptible).

The evident data gaps on trypanocide use are consistent with arguments made by Grace [26] in evaluating **antimicrobial resistance** (**AMR**), likely reflecting the limitations of low- to middle-income countries (LMICs) in surveillance capabilities for antimicrobial use [27]. Lack of reliable data on frequency and quantities of trypanocide use in AAT make it difficult to quantify the potential impact of issues such as **treatment failure** or resistance.

Effectiveness of trypanocides for treatment and prophylaxis of AAT in the field

Farmer practices in the use of trypanocides for AAT

Across SSA, most reports indicate that over 50% (range 1–90%) of farmers treat their own livestock with drugs when needed [22,28–33]. Farmers use nonspecific indicators such as body condition scoring and visual assessment to make treatment decisions [30,31] and they often do not focus on disease aetiologies [29]. Additionally, a challenge with diagnosing AAT in settings where the circulation of multiple infectious diseases [34,35] is the norm, is that many clinical signs associated with the disease are not specific to AAT [34]. In the absence of **veterinary services** or available diagnostics, it is therefore not surprising that farmers regularly misdiagnose livestock disease [30]. In one study in Kenya, it was estimated that a third of all drug treatments given to livestock were trypanocides, but 53.5% of treatments for trypanosomiasis were administered inappropriately to cattle that likely did not have AAT [36].

Farmer decision-making on type and frequency of trypanocide administration is complicated and nuanced. Clinical signs shown by the animal, farmer preferences, costs of medications, and success of their first used treatment all play a major role in choices of trypanocides [21,32]. In

work prior to entering the PCP with political and financial commitments nationally: stage 1 for capacity development and disease-risk mapping, stage 2 for reduction of AAT, stage 3 to eliminate AAT transmission, stage 4 when AAT is eliminated but control measures remain, stage 5 when AAT is eliminated and control measures are no longer practiced.

Prophylactic: any medication used to prevent a disease from occurring.

Targets: insecticide-treated panels of cloth, sometimes baited with artificial attractants, used to attract and kill tsetse flies.

Treatment failure: the situation that occurs when a treatment is applied to an infectious disease and the symptoms of the disease do not resolve and/or test results show continued infection.

Trypanotolerant cattle breeds: certain breeds of African taurine cattle that are intrinsically at a reduced risk of developing clinical symptoms with AAT as compared to trypanosusceptible breeds (indicine and European taurine breeds exotic to Africa).

Veterinary services: animal healthcare provided by trained professionals, inclusive of veterinarians, paravets, animal health technicians, and community animal health workers.

some instances farmers use multiple trypanocides [21] or a mixture of trypanocides and antibiotics [37] at the same time. Choice and frequency of trypanocides can also be influenced by the age class and working requirements of animals [38].

Farmers in Nigeria, Ethiopia, and across a multisite study in SSA reported that DZ was their first-choice treatment for AAT on 55–79% of occasions, whereas only 8–21% chose ISM as their first-line treatment [22,28,32,38]. Farmers use DZ to treat individual sick cattle, or whole herds, and in some cases, they indicate using it as a **prophylactic**, even though this medication has no prophylactic capability [13,31,38]. ISM, by contrast, is a prophylactic and is used as such but quite variably, with usage reported less frequently in Tanzania and Ethiopia (5–45% use ISM) [13,38], than in Zambia (99% use ISM) where ISM is reportedly used by farmers upwards of four times/year [31]. In Nigeria, across all trypanocide and treatment options for AAT, most are given twice seasonally [22], and in Ethiopia individual cattle are treated upwards of seven times/year [32]. Several authors conclude that farmers prefer DZ as it is less costly, single dose, and also treats *Babesia* infections, and it is the farmers' preference for treating AAT cases (DZ is for treatment) as opposed to using prophylaxis [13,32,38]; however, more robust studies of the factors driving trypanocide use are needed. No recent published data on the use of homidium salts (HM) are available, although anecdotally HM is still widely used in some areas.

Farmer-reported use has demonstrated issues with both underdosing and overdosing of trypanocides, as well as with other antimicrobials more generally [21,28,30,31]. For example, farmers in southwestern Ethiopia base trypanocide dose on age class of animal, but generally overdosed young animals (87% of DZ doses in young animals were overdoses) and underdosed adults (40% of ISM doses in adults were underdoses) [38]. Interestingly, DZ was dosed correctly 94% of the time in adult cattle, as compared with 40% in the case of ISM treatment [38]. This could be due to the higher cost of ISM, or because it is sold in a multidose sachet in some localities as opposed to DZ, which is usually sold as a single-dose sachet. In Nigeria, inappropriate dosing was due to farmers tending not to review the concentration of the trypanocide, the dose, or the animal's weight in their decision-making process [22]. The use of expired or improperly stored medication can also lead to treatment failure [13,29,39], and in Ethiopia, 56.7% of farmers did not check expiry date [39]. Language barriers and literacy can also impede reading of the expiry date and dosing recommendations on packages of medications, with instructions rarely provided in local languages.

In order to achieve optimal therapeutic range, trypanocides currently on the market are licensed to be administered by deep intramuscular (IM) injection (Box 1); however, only 68–74% of farmers correctly identified the route by which to administer medication in one study in Mali and Burkina Faso [28]. In Tanzania, up to 75% of farmers preferred the intravenous (IV) route of administration for trypanocides because they believed it to be faster-acting, whereas others opted for both IM and IV administration concurrently. Inappropriate routes of administration can lead to treatment and prophylaxis failure, and to injection-site reactions, which was noted in 24% of cases in West Africa [30] (see also Box 1).

Lack of access to diagnostics when making decisions on treatment with trypanocides

Farmers base diagnosis of AAT on clinical symptoms as they do not usually have access to diagnostic tools. Current diagnostics for AAT require costly equipment and skilled interpretation (Box 2). Given limited laboratory capacity in LMICs, these facilities are often confined to regional laboratories, constraining access of farmers and veterinary services [26]. There is limited evidence assessing the success rate of farmers diagnosing AAT correctly based on clinical signs. One small study in West Africa reported that, among farmer-diagnosed cattle, 84% had evidence of AAT on

Box 2. Commonly used diagnostic methods for AAT

Parasitological diagnosis can be achieved by wet and Giemsa-stained blood films but this has low sensitivity, especially when there are low levels of parasites in the blood, which is common in chronic infections. Trypanosomes in blood can be concentrated by the haematocrit centrifugation technique (HCT) with subsequent examination of the buffy coat/plasma junction. Sensitivity is further improved through evaluation with the buffy coat technique (BCT) in which the haematocrit tube is cut and the buffy coat/plasma layer is applied to a slide for dark-ground or phase-contrast microscopy. These methods increase sensitivity due to concentrating the parasites; however, a threshold of 2.5×10^2 parasites/ml of blood is required to detect infection via these methods. These methods can be used in the field provided a microscope and centrifuge are available.

Subinoculation of blood from suspected infectious animals can be injected into clean recipient hosts, such as immunosuppressed rodents. This method is costly, labour intensive, and requires expertise, but can be more sensitive depending on the species of trypanosome (it typically fails with *T. vivax* as very few strains grow in mice).

Immunological diagnostic methods are more sensitive and can be done by complement fixation, indirect fluorescent antibody test, card agglutination trypanosomiasis test, and ELISA (antigen and antibody). One serological test (VeryDIAG) is commercially available as a rapid diagnostic test; it targets antibodies against different *T. congolense* and *T. vivax* antigens [58]. All serological tests are relatively expensive, and all current tests are based upon antibody detection, meaning that they cannot differentiate between active and recent infection and can require technical capabilities (VeryDIAG being the only 'pen-side' test available). Molecular methods such as PCR can be used to gain species-specific diagnosis but they are expensive and require sample processing, expertise, and equipment. Potentially field-friendly isothermal PCR assays, such as LAMP, have been designed for the AAT species, but have not been developed to the point of field deployment. Whilst further pen-side diagnostics are under development, reducing the costs sufficiently to make them accessible to livestock keepers is challenging.

laboratory test [30], and in Kenya, approximately 50% of farmer diagnoses were estimated as correct for AAT based on the clinical symptoms described (but unconfirmed by laboratory testing) [36]. However, these studies are too small to draw generalizable conclusions. Decision-support tools have been proposed to assist vets and extension workers in differentiating endemic diseases of cattle in SSA on the basis of clinical presentation [40], but to our knowledge have not yet been validated against gold-standard diagnostics.

Drug quality and counterfeit drugs

Farmers across SSA have reported concerns about poor drug quality, stating that some brands are more trustworthy than others and attributing treatment failure to drug quality up to 61% of the time [21,25,29]. It is difficult for farmers to gauge drug quality, with purchasing drugs likened to a lottery [21]. Farmers cannot easily assess the performance of a drug, since treatment success is confounded by dose, method of administration, and whether the animal actually has AAT.

Farmers' concerns are legitimate with quality analysis of DZ and ISM in West Africa, Togo, and Ethiopia, indicating that 51.9%, 40%, and 28% of drugs were noncompliant (i.e., not containing the correct dose of active ingredient), respectively [32,41,42]. Compliance varied by country of sale, country of manufacture and official vs non-official sources. ISM was found to have a higher rate of noncompliance across West Africa [42] as compared to DZ, but the opposite was found in Togo [41], and no significant difference was found in Ethiopia [32]. The sample sizes in these studies were small, limiting the conclusions that can be drawn and reflecting similar challenges in relation to AMR and drug quality where anecdotal mentions in the literature exceed the few systematic studies [43].

Poor-quality drugs can arise from both illegal (i.e., counterfeit) production and substandard practices that are related to the difficulty in producing trypanocides under good manufacturing practice quality standards [21]. Counterfeiting is hard to corroborate in the literature as it is illegal; however, it is exacerbated by inadequate veterinary practice or state-enforced controls [21]. Substandard production issues are amplified by country-level regulators having limited capacity

to test, identify, and remove substandard products [21,43]. In addition, while drug monographs have been published for DZ, ISM, and HM, there is no agreed international standard for quality control, although work was started in 2014 to support this effort [19].

Resistance to DZ and ISM across SSA

Reports of treatment failure with trypanocides in SSA stretch back to at least 1991 [44]; however, trypanocide resistance can be hard to differentiate from the other causes of treatment failure, and the epidemiology and impact of trypanocide resistance remain unclear. Literature on resistance suffers from variable methodology and robustness, inconsistent diagnostic tests and reporting, small sample sizes, and is heavily focused on *Trypanosoma congolense* (Table 1). These issues are similar to those for AMR research generally in LMICs [26]. Tests for resistance in cases of AAT are limited to few options (Box 3) and involve evaluating response to treatment in animal models [45]. No reliable genetic marker for resistance to veterinary trypanocides has been verified. There are, accordingly, few publications on objectively evaluated resistance rates, and almost no literature that distinguishes true resistance from simple reinfection or treatment failure for other reasons. Although it is clear that treatment failure is common, and at least some failures are likely to relate to true parasite resistance to drugs, definitive evidence is scant and improved methods to detect and follow drug resistance are needed. Although it is often suggested that resistance is increasing over time, the lack of robust data means that inference regarding trends, risk factors, or impact of resistance is limited (Table 1 and Box 4).

Limited data on clinical cure rates and treatment failure

There are multiple reasons why treatment failure can occur, including inappropriate administration (i.e., the animal does not have AAT), incorrect dosage or route of administration, low drug quality, and resistance. However, there is limited information on clinical cure rates or the relative importance of these factors. The only relevant data stem from results of drug-resistance studies (Table 1) which often suffer from inconsistent approaches, and are rarely representative of how trypanocides are used in a field setting, and farmer-reported success rates [25,28,32,38,39,46] which are constrained by the lack of objective diagnostic tests (Boxes 2, 3). There is currently no research that the authors could find to indicate the true rate of treatment failure or cure in a field setting (farmer-treated livestock) on clinical cases of AAT diagnosed by laboratory methods.

Current influences on trypanocide use in the field

The frequency of trypanocide use varies greatly, with drivers affecting AAT exposure (such as proximity to game management areas, intensive vs. extensive management, grazing practices, seasonality), culture and beliefs (ethnic group, historical control procedures), and socioeconomic status [21,25,29,31]. Most of these drivers also affect the likelihood that drugs are used appropriately, through their influence on access to veterinary services. Across SSA, most pharmaceuticals used in livestock are administered without veterinary oversight [13,26,29,30,32,38]. The overarching reasons leading to inappropriate use of livestock medications include the high number of animals requiring treatment, scarcity of veterinarians or animal-health professionals, and lack of capacity to implement a veterinary system with appropriate oversight for prescription-based medications [26].

Whilst, historically, responsibility for AAT control largely fell to government, with state-funded tsetse-control programmes, following the transition from state to private veterinary services in the 1980s, systems began to suffer from lower-quality products, unsubsidized services/inputs, unregulated practices, and underserved communities – especially in rural areas [21]. The gap in services has been filled by farmers purchasing medication at private pharmacies or open markets, but the lack of capacity for providing animal-health services has limited oversight of

Table 1. Recent reports of resistance to diminazene aceturate and isometamidium chloride across sub-Saharan Africa[a]

Location and methods	Reports of resistance	Refs
Southwest Ethiopia - Longitudinal study of *n* = 106 positive (cases) and *n* = 119 negative (controls) cattle diagnosed by Woo haematocrit centrifuge technique. - Visited monthly for 6 months for Woo technique testing - Positive cattle at any visit or with PCV <18% were treated with DZ, and if remained positive a double dose of DZ, and if still positive a dose of ISM. - *T. congolense* samples analysed using the invalid DpnII-PCR-RFLP resistance test, hence no conclusions can be inferred from those test results	- Resistance to DZ - Four outcomes following treatment evaluated (i) new infections, (ii) relapse, (iii) combination of both, (iv) cure - Results: over 6-month monitoring of infected and uninfected cattle, 40% of events were new infections, 37.5% relapses, and 22.5% cure rate - Lack of flow diagram to identify positive animals over course of study	[38]
Zambezia province, Mozambique - Intervention study with random allocation to either ISM or DZ across three study sites - Study site 1 *n* = 31 (DZ); *n* = 34 (ISM) - Study site 2 *n* = 10 (DZ); *n* = 11 (ISM) - Study site 3 *n* = 10 (DZ); *n* = 10 (ISM) - Monitored day 0, 14, 28 post-treatment, then interventions swapped between groups and monitored for a further 14 days - Outcome is blood test results by BCT and PCR - Day 14 and 28 results indicative of drug resistance - Day 42 results indicative of multidrug resistance	- Resistance to DZ and ISM - Results: evaluation of cattle following treatment by combined BCT and PCR test results - Site 1 had 23% and 26% positive animals in the DZ treatment group at days 14, 28 - Site 1 had 14% and 14% positive animals in the ISM treatment groups at days 14, 28 - Site 2 had 50% positive animals in the DZ treatment groups at days 14 and 28 - Site 2 had 36% and 18% positive animals in the ISM treatment groups at days 14, 28 - At day 42, site 1, 12% animals tested positive, and at site 2 a total of 19% animals tested positive - Site 3 had no reports of positive cases following treatment - Unclear if positive animals at each stage were the same animals or new animals (reinfection vs. resistance unclear)	[46]
Nigeria - One herd (*n* = 79) of cattle in Nigeria where 54.4% (*n* = 43) of cattle were positive based on microscopy for AAT were treated with DZ and tested by ITS-PCR 2 months post-treatment	- Resistance to DZ - Results: evaluation of cattle herd following treatment - 19/79 cattle were positive 2 months following treatment - Unclear if 19 parasite-positive cattle were cattle parasite-positive at day 0 and 2 months post-treatment (reinfection vs. resistance unclear)	[64]
Northern Togo - Intervention study with two groups of positive cattle; one treated with DZ (*n* = 50) and other with ISM (*n* = 50) - monitored by PCV and BCT at day 14 and 28 post-treatment - DpnII-PCR-RFLP DZ resistance testing of positive *T. congolense* samples from the intervention study and an earlier cross-sectional study using the invalid DpnII-PCR-RFLP resistance test, hence no conclusions can be inferred from these results	- Resistance to DZ and ISM - Results: in the DZ-treated animals, there was a 14% failure rate on day 14 overall - In the ISM-treated animals, there was a cumulative failure rate (days 14 and 28) of 26% - Unclear if positive animals at each stage of testing were the same animals or new animals testing positive (reinfection vs. resistance unclear)	[14]
Southeast Mali - Intervention study with two groups of positive cattle; one treated with DZ (*n* = 62) and the other with ISM (*n* = 63) - Monitored at days 14 (DZ and ISM) and 28 (ISM) for PCV and trypanosomes by dark-ground phase-contrast microscopy	- Resistance to DZ and ISM - Results: at day 14, (32%) of cattle were still positive following treatment with ISM - Of the 43 cattle that were negative at day 14 in the ISM group, 11 (26%) were positive at day 28 - *T. congolense* accounted for 77% of all ISM treatment failures	[65]

(continued on next page)

Table 1. (continued)

Location and methods	Reports of resistance	Refs
	- At day 14 following treatment in the DZ group, 31% remained positive and retreatment with a double dose of DZ still led to treatment failure in 26% of cases - *T. congolense* accounted for all of the DZ treatment failures	
Northwest Ethiopia - Intervention study where purchased cattle (*n* = 24) were divided into four equal groups; half were exposed to *T. vivax* from tsetse-infested areas, and half with *T. vivax* from non-tsetse areas - Exposure was via intravenous inoculation - Then within each exposed group either ISM or DZ was given (2 DZ groups and 2 ISM groups) at peak parasitaemia - Cattle evaluated by physical exam, PCV, the Murray method of microscopy (BCT) twice a week for 100 days - If relapse of infection occurred the treatment with a different drug than first used was initiated - Treatment considered successful if negative following treatment with one or two doses of trypanocides given - Relapse considered if trypanosomes detected following trypanocidal drug administration	- Resistance of *T. vivax* to DZ and ISM - Results: following drug administration in all cattle, parasitaemia significantly reduced within 24 h - 9 (37.5%) cattle showed relapsing infections, 4 from the non-tsetse-infested *T. vivax*, 2 from ISM and 2 from DZ; and 5 from the tsetse-infested *T. vivax*, 3 from ISM, and 2 from DZ - relapses began 35 and 56 days post-treatment across all 9 animals	[66]

[a]Abbreviations: BCT, buffy coat technique; DpnII-PCR-RFLP, diagnostic test aimed to evaluate for resistance gene for DZ, but subsequently shown to be spurious since the gene evaluated (TcoAT1) is not involved with drug resistance; DZ, diminazene aceturate; ISM, isometamidium chloride; PCR, polymerase chain reaction; PCV, packed cell volume.

the market, and left many farmers, particularly in rural areas, with no animal-health support or advice. The limited services that are available in many areas are not always utilized by farmers for reasons of mistrust, preference for local traditional medicines, cost, or a perceived lack of benefit if they believe that they can do the same job themselves [13,47,48]. In one study in Tanzania, 82.3% of farmers reported that they did not request veterinary services but instead used medication or local remedies themselves [13]. The trends in Tanzania show that, in more densely populated urban areas with smaller intensive/mixed farming used for market sales, having a greater knowledge of AAT, and being in a high-burden area, led to the greater use of veterinary services [29]. But across five SSA countries where there are large, extensively managed trypanosusceptible cattle herds in high-risk areas, and where farmers were more likely to self-diagnose and treat their cattle, the farmers were less likely to consult animal-health professionals [25]. In the absence of other animal-health services, farmers commonly rely on trial-and-error methodology, and seeking advice from friends, family, neighbours, and small-scale retailers with whom they have built trusted relationships [13,21,29,33,49].

This scenario has led to a proliferation of drug manufacturers and distributors, as well as informal markets, through which farmers directly purchase drugs in the absence of veterinary oversight [13,21,31,32]. In Ethiopia, two studies found that 56% and 33.9% of farmers obtained their medication from the unauthorized market or illegal drug market, respectively [32,47]. More specifically to trypanocides, one Ethiopian study reported that 97.5% of farmers obtain these drugs from informal pharmacies [39] which offer limited education or services to farmers [50]. The education level of the staff in veterinary pharmacies and drug shops or agrovets is variable, with only 56% having attained higher education degrees, and the condition of the shops in relation to drug storage and handling was rated as fair or poor in more than one half of the shops visited in Ethiopia [39].

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Box 3. Diagnostic methods for resistant infections of AAT

Drug resistance can be detected via inoculation and treatment tests in ruminants and mice. *In vitro* testing has been difficult to achieve with *T. congolense*, and adaptation of *T. vivax* field isolates to culture is not yet possible. Clearly, development of *in vitro* systems for cultivating bloodstream forms of both parasites will be of significant value. Tests in ruminants occur on infected animals whereby they are treated with a trypanocide and monitored for cure or treatment failure over 100 days in a laboratory setting where there is no risk of reinfection. This is costly and labour intensive to perform and does not reflect field settings. Block treatment studies and longitudinal studies on parasitological data can be done in the field on ruminants. This involves analysing the number of infections following a block (group) treatment to animals and comparing the number of infections in an untreated group. The longitudinal data collected can be used to compare incidence and prevalence with varying treatment regimes.

Mice can be similarly infected, with data on the curative dose in a proportion of mice, and a single dose approach has been developed to enable relatively objective analysis of resistance and diminish the costs of testing [45]. However, this still requires facilities and expertise to be available, many strains of *T. vivax* do not grow in mice, and the results in mice do not always correlate to cattle.

Trypanocidal drug ELISAs in combination with parasite detection can be used together to detect resistant trypanosomes [59,60]. When trypanosomes are present at specified concentrations of trypanocides, one can deem it a resistant infection. However, there are issues with variability of pharmacology of trypanocides in cattle that can lead to inaccurate results.

Molecular tests seeking resistance-related genes have not so far been reliably developed for AAT. One test purporting to detect resistance to DZ, based on different forms of a gene encoding transporter (TcoAT1) [61], does not in fact reveal resistance. The transporter encoded by the gene does not transport DZ, and any link between different forms was coincidental [62]. Studies using this test should be disregarded. It is hoped that the identification of genes apparently linked to ISM resistance [63] might offer routes to genetic tests if validated. Loss of genes encoding CBP1 serine caboxypeptidase [56] offers a possible route to genetic testing for the benzoxaborole class if they are marketed for use.

Regardless of the diagnostic method used, the cost must be a consideration as it must be accessible to settings in SSA, and ideally less costly than the treatment for AAT.

Certain ethnic groups living in more rural, less-developed areas, and with greater exposure to wildlife-protected areas, are often blamed for their inappropriate (administering drug without diagnosis of causative agent, or incorrect administration) use of livestock medications [21,29]. These farmers are acutely aware of the problems but are systemically excluded from veterinary care and therefore have no other choice but to make treatment decisions on their own. Farmers, and those from whom they seek advice, suffer from a lack of knowledge on AAT and often have low levels of education [22,39]. However, farmers are willing to pay for animal-health services when they are available and trusted, and imaginative solutions are needed to improve service provision within the existing resource constraints to allow farmers to use trypanocides more effectively.

Interventions at local, national, and international levels to improve the effective use of trypanocides

Whilst several international or large-scale regional AAT-control programmes have been instigated over the past few decades, most have focused predominantly on tsetse control, with no large-scale strategies for optimizing the use of trypanocides as part of AAT control. A **progressive control pathway** (**PCP**) has been outlined for AAT [51]. The issue of wildlife reservoirs, and lack of sustainable methods to eradicate tsetse, renders eradication of AAT an unlikely goal, but a logical and disciplined framework offers the possibility of incremental progression towards the challenge. Whilst the PCP acknowledges the role of trypanocides, the later stages (3–5) rely on large-scale control of tsetse. However, most countries endemic for AAT are in the early stages [7] and livestock keepers are heavily dependent on trypanocides. Further research and guidance to promote effective and sustainable use of trypanocides would be beneficial to help inform national and international strategies.

Paired with national and international programmes, community engagement is essential to mitigate AMR. Methods suggested include enhanced approaches at community-level education, including better public health messaging via veterinary services, use of radio to reach more rural

Box 4. Improving reporting of control practices of AAT in field studies

Epidemiologic studies on AAT are typically cross-sectional, with farmer-reported results providing data of limited resolution and value. Another common study type has involved evaluating drug resistance through interventional trials. Interventional studies evaluating resistance through administration of trypanocides to infected animals to test treatment failure are often hampered by lack of clarity on whether animals are persistently infected or reinfected, given difficulties in following individual animals over time within a given study. This makes it unclear if there is genuine resistance or if there is a high risk of reinfection. These studies also use varying methodologies, and particularly use different diagnostic approaches with varying sensitivities and specificities, making them difficult to compare. This lack of robust epidemiologic data on AAT control interventions makes it difficult to implement evidence-based decisions about AAT control.

We suggest three routes to improve consistency and reporting in AAT epidemiology studies:

(i) Standardized reporting methods for epidemiologic studies should be used. In trials, the Consolidated Standards of Reporting Trials (CONSORT) check list can be used, whereas in observational studies the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) check list can be used. Consultation with an epidemiologist and/or statistician should be considered if one is not already part of the research team.
(ii) When reporting, special attention should be paid to sampling size and strategy, eligibility, refusal rate for participation, sample size contributing to reported statistics, descriptive data on study participants, measurement of reported data (farmer or investigator), handling of missing data, study dates, randomization and blinding in interventional studies, and reporting full statistical models instead of partial ones.
(iii) Standard operating procedures should be developed for common methodologies to improve comparability between studies. When developing these, attention should be paid to ensuring that protocols are as practical as possible for field scenarios or areas with limited infrastructure.

areas, bridging cultural disconnects, and relationship building [21,29]. However, training alone is found to be ineffective unless it is partnered with incentives, accountability, and a sustainable plan for local ownership and responsibility [25,26].

Implications of the current use of trypanocides in the field for new drug development and deployment

The presence of resistance to existing drugs suggests that new drugs will be an essential pathway to manage AAT; however, counterfeit or poor-quality drugs on the market disincentivize the legitimate market for currently available and new trypanocides [21]. Work is ongoing to evaluate combined drug therapy [52], and novel trypanocides [53], with the most promise shown by the benzoxaborole class [54]. Benzoxaboroles appear to inhibit the trypanosome CPSF3 protein, involved in mRNA processing [55], a mode of action distinct from other trypanocides, diminishing the risks of cross-resistance. The leading veterinary class of benzoxaboroles can accumulate at very high concentrations in the parasite because the parent compound (e.g., AN11736) is a prodrug that is activated by enzymatic cleavage [56]. However, *T. brucei* and *T. congolense* can lose the genes that encode the prodrug-activating enzyme, rendering the drug less effective and leading to resistance. *Trypanosoma vivax* is likely to use the same mechanism, rendering it equally susceptible to this issue [56].

Trypanocides currently in use were developed during the early postcolonial period. Subsequently, the merger of international pharmaceutical companies, and increased focus on markets with higher profit margins, has led to the neglect of the needs of relatively poor farmers in Africa. However, things are changing. The Global Alliance for Livestock Veterinary Medicines (GALVmed) was founded in 2011 to address failure in the provision of new products for animal health in LMICs, and GALVmed brought forward the benzoxaborole class, initially discovered by Anacor Pharmaceuticals [57]. Pharmaceutical companies are increasingly involved in initiatives to improve farmers' access to animal-health drugs[ii]. It appears, therefore, that after a 50-year hiatus, opportunities to reinvigorate animal health care in Africa are emerging. However, pharmaceutical companies may lack awareness of consumer demands, needs, and drivers for trypanocide use, and efforts are still needed to bridge the gap between pharma and farmer.

Concluding remarks

Farmers are heavily dependent on trypanocides and invest substantial funds to control AAT. New drug development is an essential component in managing AAT; however, there are serious problems which need to be addressed regarding current trypanocide use in order for new drugs to be used effectively in a field setting. At this time, the volume and types of trypanocides being used across all livestock species, and at what cost, is unclear. The sources of these medications, and their quality, also remain mired with concerns of quality and counterfeiting. Publications on trypanocide resistance lack comparability and clarity on instances of resistance vs. reinfection, or treatment failure due to other issues around drug administration. There are no publications that the authors could find on the rate of treatment failure using accurate diagnostic tests with farmer-administered medications. Research on AAT needs to meet higher epidemiological quality standards through the use of more standardized protocols, as well as the use of CONSORT, STROBE (Box 4), or similar checklists to ensure that data are of high quality and comparable.

Farmer education, lack of affordable and easy-to-use diagnostics for AAT, and inappropriate use of trypanocides must all be addressed to ensure that both current and new drugs can be used appropriately in the control of AAT. Control programmes need to be sustainable at the local, national, and international level, and must be standardized. Funding is a key component once there is cross-sectoral commitment to these programmes, but major behavioural change is required. These all remain challenges as there is no clear leader or success story to emulate or learn lessons from (see Outstanding questions).

Outstanding questions

What is the baseline level of AAT and associated use of trypanocides in field settings across SSA in all livestock species?

What quantities of trypanocides are being used per year across SSA, and at what cost to the farmer?

What are the sources of trypanocides being used in SSA, and what is their quality standard?

What are the true rates of treatment failure in cases of AAT (due to drug administration, handling, and quality) and trypanocide resistance in different field settings?

Can low-cost diagnostic methods be introduced to detect AAT in the field and diagnose resistant infections?

How can international, national, and local investment create sustainable and integrated methods to use trypanocides and control AAT?

How can behavioural changes in farmers with limited access to animal-health services be fostered to allow sustainable administration of medications to livestock?

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Declaration of interests

The authors declare no competing interests.

Resources

[i]www.fao.org/news/story/en/item/123165/icode

[ii]https://ng.zoetis.com/zoetis-a.l.p.h.a-initiative.aspx

[iii]https://in.virbac.com/home/product-selector/pagecontent/find-the-right-product/antrycide.html

[iv]https://gloriaexports.com/product/glorotryp-2/

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